

WHAT IS CLAIMED IS:

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Sub Q18
1. A method of producing a differentiated antigen presenting cell (APC), the method comprising: culturing a population of peripheral blood or bone marrow mononuclear cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, palmitic acid for a sufficient time to produce the differentiated antigen presenting cell.

10 2. The method of claim 1, wherein the differentiated APC is a dendritic cell.

3. The method of claim 2, wherein the dendritic cell produces substantially no IL-12.

4. The method of claim 3, wherein the dendritic cell produces IL-10.

5. The method of claim 4, wherein the dendritic cell is an mDC2.

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6. The method of claim 2, wherein the dendritic cell is a CD1a⁺ dendritic cell.

7. The method of claim 6, wherein the CD1a⁺ dendritic cell is capable of presenting an antigen to a T cell.

8. The method of claim 1, wherein the population of mononuclear cells is derived from a human or a non-human animal.

20 9. The method of claim 1, further comprising depleting the population of mononuclear cells of T, B and NK cells.

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10. The method of claim 9, comprising depleting the population of mononuclear cells with immunomagnetic beads.

25 11. The method of claim 1, comprising deriving the population of mononuclear cells by density gradient separation of standard buffy coat preparations of peripheral blood.

12. The method of claim 11, further comprising depleting the population of mononuclear cells of T, B and NK cells.

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13. The method of claim 12, comprising depleting the population of mononuclear cells with immunomagnetic beads.

5 14. The method of claim 1, wherein the population of peripheral blood or bone marrow mononuclear cells comprises monocytes.

15. The method of claim 1, wherein the culture medium comprises Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid.

10 16. The method of claim 15, wherein the culture medium further comprises approximately 0.25% (w/v) bovine serum albumin and between about 1.5 and 2 mg/L 2-amino ethanol.

17. The method of claim 15, wherein the dendritic cell is a CD1a⁺ dendritic cell.

18. The method of claim 17, wherein the dendritic cell substantially lacks IL-12 production.

19. The method of claim 18, wherein the dendritic cell has substantially increased IL-10 production as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

20. The method of claim 6 or 17, wherein the CD1a⁺ dendritic cell induces or promotes Th0/Th2 differentiation of T cells.

21. The method of claim 1, wherein the culture medium comprises Yssel's medium.

22. The method of claim 21, wherein the Yssel's medium further comprises about 10% fetal bovine serum, about 2 millimolar (mM) glutamine, about 50 Units/milliliter (U/ml) penicillin and about 100 micrograms/milliliter (μg/ml) streptomycin.

23. The method of claim 21, wherein the differentiated APC is a dendritic cell.

24. The method of claim 23, wherein the dendritic cell is a CD1a⁺ dendritic cell.

25. The method of claim 23, wherein the dendritic cell substantially lacks IL-12 production or induces or promote differentiation of T cells to Th0/Th2.

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26. The method of claim 23, wherein the dendritic cell has substantially increased IL-10 production as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

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~~27. The method of claim 23, wherein the CD1a⁻ dendritic cell induces or promotes Th0/Th2 differentiation of T cells.~~

~~28. The method of claim 21, further comprising culturing the APC in the presence of an anti-CD40 monoclonal antibody for a period of approximately 24 hours, thereby providing an activated APC; and culturing the activated APC in the presence of lipopolysaccharide (LPS) and interferon-gamma (IFN- γ) for a period of approximately 48 hours, thereby producing a mature CD83⁺, CD1a⁻ dendritic cell.~~

~~29. The method of claim 28, wherein the CD83⁺, CD1a⁻ dendritic cell substantially lacks production of IL-12.~~

~~30. The method of claim 6 or 24, further comprising introducing to at least one CD1a⁻ dendritic cell at least one exogenous DNA sequence operably linked to a promoter that is capable of controlling expression of said DNA sequence, which at least one exogenous DNA sequence encodes at least one antigen, in an amount sufficient that expression and presentation of the at least one antigen results, thereby producing an antigen presenting CD1a⁻ dendritic cell.~~

~~31. The method of claim 30, further comprising introducing said at least one exogenous DNA sequence to at least one CD1a⁻ dendritic cell by a method selected from electroporation, injection, microinjection, gene gun delivery, lipofection, DOTAP supplemented lipofection, DOSPER supplemented lipofection, or Superfection.~~

~~32. The method of claim 6 or 24, further comprising introducing a sufficient amount of at least one antigen or fragment thereof to at least one CD1a⁻ dendritic cell, such that presentation of the at least one antigen on at least one CD1a⁻ dendritic cell occurs, thereby producing an antigen presenting CD1a⁻ dendritic cell.~~

~~33. A differentiated antigen presenting cell (APC), which differentiated APC does not express CD1a cell surface marker.~~

~~34. The differentiated APC of claim 33, wherein said differentiated APC comprises a monocyte-derived CD1a⁻ dendritic cell.~~

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35. The differentiated APC of claim 34, wherein monocyte-derived CD1a⁺ dendritic cell substantially lacks IL-12 production.

36. The differentiated APC of claim 34, wherein the monocyte-derived CD1a⁺ dendritic cell induces or promotes differentiation of T cells to Th0/Th2 subtypes.

10 37. The differentiated APC of claim 34, wherein the monocyte-derived CD1a⁺ dendritic cell is produced by culturing a population of monocytes in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid.

38. The differentiated APC of claim 37, wherein the culture medium comprises Yssel's medium.

39. The differentiated APC of claim 37, wherein the monocyte-derived CD1a⁺ dendritic cell has substantially increased IL-10 production as compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

40. The differentiated APC of claim 34, wherein the monocyte-derived CD1a⁺ dendritic cell comprises an mDC2.

41. The differentiated APC of claim 34, wherein the monocyte-derived CD1a⁺ dendritic cell has a transfection efficiency greater than that of a dendritic cell produced by culturing a population of monocytes in IL-4, GM-CSF, and a culture medium comprising RPMI.

25 42. A method of inducing in a subject an immune response to at least one antigen, said method comprising administering to the subject a population of CD1a⁺ dendritic cells, said CD1a⁺ dendritic cells presenting at least one of said at least one antigen, in an amount sufficient to induce the immune response to said at least one antigen.

30 43. The method of claim 42, wherein said CD1a⁺ dendritic cell substantially lacks IL-12 production.

44. The method of claim 42, wherein said CD1a⁺ dendritic cell is produced by culturing a population of peripheral blood or bone marrow mononuclear cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium

comprising insulin, transferrin, linoleic acid, oleic acid, palmitic acid for a sufficient time to produce the differentiated antigen presenting cell.

45. The method of claim 42, wherein the subject is a human or a non-human animal.

46. A method of inducing differentiation of T cells, the method comprising: co-culturing a population of T cells with population of CD1a⁺ antigen presenting cells (APC), thereby inducing or promoting differentiation of said T cells.

47. The method of claim 46, wherein the T cells comprise naïve T cells.

48. The method of claim 46, wherein the antigen presenting cell is a CD1a⁺ dendritic cell.

49. The method of claim 48, wherein the CD1a⁺ dendritic cell produces substantially no IL-12.

50. The method of claim 3 or 48, wherein the dendritic cell produces substantially no IL-12 compared to a dendritic cell produced by culturing a population of peripheral blood or bone marrow mononuclear cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

51. A differentiated T cell produced by the method of claim 46.

52. A composition comprising CD1a⁺ dendritic cells.

53. The composition of claim 52, wherein said CD1a⁺ dendritic cells are capable of presenting an antigen to a T cell.

54. The composition of claim 52, wherein said CD1a⁺ dendritic cells produce substantially no IL-12.

55. The composition of claim 52, wherein said CD1a⁺ dendritic cells promote differentiation of T cells to a Th0/Th2 subtype.

56. The composition of claim 52, wherein said CD1a⁺ dendritic cells display or present at least one antigen or antigenic fragment thereof.

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57. The composition of claim 56, wherein the at least one antigen or antigenic fragment comprises a protein or peptide differentially expressed on a cell selected from the group consisting of a tumor cell, a bacterially-infected cell, a parasitically-infected cell, and a virally-infected cell, a target cell of an autoimmune response.

58. The composition of claim 52, wherein the composition comprises a vaccine.

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59. The composition of claim 52, further comprising a pharmaceutically acceptable carrier.

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60. A method of inducing or modulating an immune response in an immunocompromised subject, said method comprising administering to the subject a population of CD1a⁺ dendritic cells in an amount sufficient to induce or modulate an immune response in the subject.

61. An ex vivo method of inducing in a subject a therapeutic or prophylactic immune response against at least one antigen, the method comprising:

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a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of dendritic cells comprising CD1a⁺ dendritic cells;

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b) introducing to the population of CD1a⁺ dendritic cells a sufficient amount of at least one antigen, or a sufficient amount of an exogenous DNA sequence operably linked to a promoter that controls expression of said DNA sequence, said DNA sequence encoding at least one or said at least one antigen, such that the presentation of the antigen on the CD1a⁺ dendritic cells results; and

c) administering the antigen-presenting CD1a⁺ dendritic cells to the subject in an amount sufficient to induce a therapeutic or prophylactic immune response against said at least one antigen.

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62. The method of claim 61, wherein the culture medium comprises Yssel's medium.

63. A method for therapeutically or prophylactically treating a disease in a subject suffering from said disease, the method comprising:

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a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM)

5 supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of CD1a⁺ dendritic cells;

b) introducing to the population of CD1a⁺ dendritic cells a sufficient amount of at least one disease-associated antigen, or a sufficient amount of an exogenous DNA sequence operably linked to a promoter that controls expression of said DNA sequence, said DNA sequence encoding at least one of said at least one disease-associated antigen, such that presentation of the disease-associated antigen on the CD1a⁺ dendritic cells results; and

c) administering a therapeutic or prophylactic amount of the CD1a⁺ dendritic cells presenting the disease-associated antigen to the subject to treat said disease.

15 64. The method of claim 63, wherein the culture medium comprises Yssel's medium.

65. A method for therapeutically or prophylactically treating a disease in a subject suffering from the disease, the method comprising:

a) culturing a population of monocytes obtained from the subject with IL-4, GM-CSF, and a culture medium comprising Iscove's Modified Dulbecco's Medium (IMDM) supplemented with insulin, transferrin, linoleic acid, oleic acid and palmitic acid for a sufficient time to produce a population of CD1a⁺ dendritic cells;

b) contacting the population of CD1a⁺ dendritic cells with a population of diseased cells from a tissue or organ of the subject, thereby inducing presentation of a disease-associated antigen on the CD1a⁺ dendritic cells; and

25 c) administering a therapeutic or prophylactic amount of CD1a⁺ dendritic cells presenting the disease-associated antigen to the subject to treat the disease.

66. The method of claim 63, wherein the culture medium comprises Yssel's medium.

67. The method of claim 63, wherein the disease is a cancer.

30 68. A monocyte-derived dendritic cell, wherein the dendritic cell does not express a CD1a cell marker, substantially lacks IL-12 production, produced IL-10, and promotes Th0/Th2 lineage differentiation of T cells.

69. A monocyte-derived dendritic cell produced by culturing a population of monocyte cells in interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), and a culture medium comprising insulin, transferrin, linoleic acid, oleic acid, and palmitic

- 5 acid, wherein the monocyte-derived dendritic cell has an altered cytokine profile compared to a dendritic cell produced by culturing a population of monocyte cells in IL-4, GM-CSF, and a culture medium comprising RPMI.

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